

## EDITORIAL

# From malignant mutations to malignant domains: the continuing search for prognostic significance in the mutant genes causing hypertrophic cardiomyopathy

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The genetic causes of hypertrophic cardiomyopathy are diverse and thus present challenges in the development of genetic tests to identify patients at risk

## GROUPING MUTATIONS ACCORDING TO FUNCTIONAL DOMAIN

In a recent issue of *Heart*, Woo and colleagues<sup>15</sup> used their knowledge of the structure and function of myosin to assess the relation between clinical outcome from HCM and disease-causing mutations clustered in specific functional domains. The authors screened the first 23 (of 38) protein coding exons of *MYH7* in 70 largely consecutive HCM patients. Eleven distinct mutations were identified in 15 probands. Next, they analysed the clinical and genetic status of family members of those patients identified with *MYH7* mutations, and then related prognosis to the functional domain affected by the *MYH7* mutation in 74 genetically affected probands and relatives.

Specifically, the 11 missense mutations were classified according to affected functional domain (active site for ATP hydrolysis, actin binding site, or head-rod junction) as well as to the type of amino acid substitution—that is, conservative (maintaining the same charge on the residue) versus non-conservative (altering the charge). Two subgroups of mutations emerged from the analysis with significantly reduced survival: non-conservative mutations of the ATP hydrolysis active site, and non-conservative mutations of the head-rod junction. Therefore, the data of Woo and colleagues<sup>15</sup> suggest that HCM patients with mutations affecting two specific functional domains are at increased risk for adverse outcome, and the specific functional domain affected by an *MYH7* mutation could be regarded as a potential risk marker.

Conceptually, it is reasonable that distinct mutations affecting different amino acid residues but leading to the same in vitro functional defect (for example, increased rate of ATP hydrolysis by myosin) would cause similar disease outcome (notwithstanding genetic and environmental modifiers). Woo and colleagues<sup>15</sup> present the first data supporting the hypothesis that grouping mutations according to functional domain may aid in assessing prognosis. Given that the dynamic interactions of the sarcomere occur via the electrostatic charges of the residues, it is also a reasonable assertion that mutations affecting those charges may culminate in a more severe phenotype and adverse outcome.<sup>2-8</sup> However, considering the myriad of possible contributing factors in a heterogeneous disease such as HCM,<sup>1</sup> including modifier genes and environmental influences, it is notable that the

**H**ypertrophic cardiomyopathy (HCM) is a disease of profound clinical heterogeneity.<sup>1</sup> Presentation and clinical course range from normal longevity with no or few symptoms, to severe disability at any age, or sudden cardiac death most commonly in the young. The genetic causes of HCM are diverse, with well over 200 mutations in 10 sarcomeric genes reported for this autosomal dominant disease.<sup>1</sup>

Research efforts have focused on correlating the genetic causes of HCM with the expressed phenotypes and prognosis, with the aspiration of formulating a genotype based risk stratification strategy. Specifically, in the  $\beta$  myosin heavy chain gene (*MYH7*), certain mutations were associated with normal life expectancy, whereas others were reported to decrease survival because of sudden arrhythmic death or heart failure.<sup>2-7</sup> Such mutations in *MYH7* and in other HCM-causing genes have been designated in the literature as either “benign” or “malignant”. It has also been suggested that charge-changing amino acid substitutions may be associated with more severe disease.<sup>2-8</sup> However, by analysing a large consecutively accessed cohort of unrelated individuals, we have reported that mutations previously designated as “benign” or “malignant” are particularly uncommon in the HCM population and are also of uncertain prognostic significance.<sup>9-10</sup> Nevertheless, identification of high risk HCM patients has recently become particularly pertinent, given the application of the implantable cardioverter-defibrillator to this disease for the primary prevention of sudden death.<sup>11-12</sup>

The  $\beta$  myosin heavy chain is a component of the sarcomere with well characterised structure and function.<sup>13</sup> Within the cardiac muscle cell, hydrolysis of ATP by the globular myosin head causes movement of myosin filaments relative to actin. As a result of this “ratcheting” motion of myosin, the sarcomere shortens, and heart muscle contracts. The specific amino acid residues that bind ATP and actin have been defined, as well as those at the flexible head-rod junction. HCM-causing mutations are clustered around these three sites, as well as in the binding site for the essential light chain and in the rod portion of the protein filament.<sup>13-14</sup>

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location and charge effect of a disease-causing mutation proved to be of prognostic significance.

## MOLECULAR PROGNASTICATION: IMPACT OF FINDINGS

The observations of Woo and colleagues<sup>15</sup> provide a potentially important and clinically relevant strategy to overcome the limitations afforded by the genetic heterogeneity of HCM.<sup>1-10</sup> Because of the diversity of pathogenic HCM mutations, and the rarity of each individual mutation in the population, it is difficult to achieve statistical significance when comparing one causative mutation to another. By grouping mutations according to the functional site affected and the effect on amino acid charge, the authors were able to make significant comparisons across subgroups. Furthermore, this strategy of grouping mutations by functional domain may prove to be valuable not only in HCM, but also in other familial diseases where genetic diversity causes the incidence of each individual mutation to be low. For example, in long QT syndrome, a similar approach of grouping for prognostic assessment has been exploited. Patients with LQT2-causing mutations localised to the pore domain have a less favourable prognosis than those with LQT2-causing mutations residing in the cytoplasmic C-terminus.<sup>16</sup>

The precise role of estimating prognosis by affected functional domain (as elegantly shown by Woo and colleagues<sup>15</sup>) awaits confirmatory studies, owing to the relatively small size of the study population, which implicitly limits the authors' ability to generalise their findings to the overall HCM population. Indeed, each of the subgroups defined was represented by relatively few families—for example, the subset with non-conservative rod domain mutations comprised only seven relatives from three families. Furthermore, all three sudden cardiac deaths occurred within one of these three families. In this context, the effect of an unknown risk factor in a family, genetic or environmental, could importantly affect the analysis and conclusions. Also, Woo and colleagues<sup>15</sup> limited their screening of *MYH7* to the first 23 exons. However, up to 20% of *MYH7* mutations may lie in the rod portion of the protein, and about 3% of HCM families harbour multiple disease-causing mutations in the same or different genes.<sup>14</sup> Therefore, the possibility remains that in some of the probands studied, a second *MYH7* mutation was linked to the identified mutation.

Finally, clinical and genetic studies in HCM generally rely on cohorts which unavoidably have been assembled with some selection bias. To balance the data from the 15 probands with *MYH7*-HCM, Woo and colleagues<sup>15</sup> recruited the participation of 148 family members. However, the motivation for participation on the part of the relatives may have been related in part to perceived severity of disease. For example, asymptomatic family members (including some previously undiagnosed) are less likely to volunteer for such genetic testing, while symptomatic or high risk relatives are more likely to become involved. As a result, statistical significance for risk factors conveying adverse prognosis will more often be achieved for mutations found in families with generally poor outcome. Therefore, this impressive data of Woo and colleagues<sup>15</sup> must be replicated with greater

numbers of families and more complete genetic screening to enhance the promise and potential application of functional domain based risk stratification to the clinical identification of at-risk HCM patients. In turn, such high risk patients with this complex and heterogeneous disease may benefit from lifesaving interventions such as implantable cardioverter-defibrillator therapy for primary prevention of sudden death.

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